Data Evaluation Report on the acute toxicity of ATSA, a pyroxsulam (XDE-742) metabolite, to fresh water invertebrates - *Daphnia* sp.

PMRA Submission Number 2006-4727; ID 1283192 EPA MRID Number 469084-27 APVMA ATS 40362

Data Requirement:	PMRA DATA CODE:	9.3.2
	EPA DP Barcode:	D332116
	OECD Data Point:	IIA 8.3.1.1
	EPA Guideline:	FIFRA 72-2 (OPPTS 850.1010)

Test material: **ATSA metabolite of pyroxsulam** **Purity (%):** 100%

Common name: ATSA Metabolite of pyroxsulam (ATSA metabolite of XDE-742)

Chemical name: 3-pyridinesulfonamide, N-(5-amino-1H-1,2,4-triazol-3-yl)-2-methoxy-4-(trifluoromethyl)

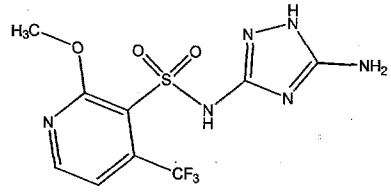
IUPAC: Not provided

CAS name: Not provided

CAS No.: Not provided

Synonyms: X11265218

Chemical structure:



Primary Reviewer: Daryl Murphy D. Murphy 22/02/08 **Date:** 14 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewer(s): J Holland 22/2/08 **Date:** 14 March 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

Thomas Steeger, Ph.D., Senior Biologist T. Steeger 4/13/08 **Date:** 20 March 2007
U.S. Environmental Protection Agency, EFED, ERB 4

Catherine Evans **Date:** 29 June 2007
Environmental Assessment Directorate, PMRA Louise Lavine for Catherine Evans 05/03/08

Company Code: DWE
Active Code: JUA
Use Site Category: 13, 14
EPA PC Code: 108702

CITATION: Marino, T. A. Arnold, B. H. Najar, J. R. and Sushynski, J. M. 2006. ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Daphnid, *Daphnia magna.* Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Project Study ID 061005. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 15 March 2006. Unpublished report.

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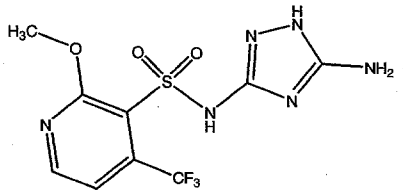
IUPAC:	Not provided
CAS name:	Not provided
CAS No.:	Not provided
Synonyms:	X11265218

Chemical structure:



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Australian Government Department of the Environment and Water Resources

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EXECUTIVE SUMMARY:

The 48 hour acute toxicity of the ATSA metabolite of pyroxsulam to *Daphnia magna* instars was studied under static conditions in a limit test. Daphnids were exposed to nominal concentrations of 0 (control) and 120 mg ATSA metabolite of pyroxsulam/L. Mean-measured concentrations were 0.252 and 121 mg/L ATSA metabolite of pyroxsulam/L . Observations of immobilisation and sub-lethal effects (behaviour or appearance) were conducted at 24 hours and at test termination. Immobilisation and sub-lethal effects were not observed in either the control or test solutions. The 48 hour EC_{50} for immobilisation and sub-lethal effects were both >121 mg ATSA metabolite of pyroxsulam/L based on mean, measured concentrations. The 48 hour NOECs, based on immobilization and on sub-lethal, were both 120 mg ATSA metabolite of pyroxsulam/L also based on mean, measured concentrations.

Based on the results of this study, the ATSA metabolite of pyroxsulam would be classified as practically non-toxic to *D. magna* instars (48 hour EC_{50} >100 mg /L) in accordance with the acute toxicity classification systems of the Australian Government Department of the Environment and Water Resources.

This study is classified as acceptable and satisfies the guideline requirements for a 48 hour acute limit toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age:	*Daphnia magna* instars of <24 hours age at the start of the exposure period.
Test Type:	Static, 48 hours
48 hour EC_{50} (immobilisation or sub-lethal effects):	>121 mg ATSA metabolite of pyroxsulam/L (mean measured concentration).
Probit Slope and 95% C.I.:	Not applicable in a limit test.
48 hour NOEC (immobilisation and sub-lethal effects):	121 mg ATSA metabolite of pyroxsulam/L (mean, measured concentration).
Endpoint(s) Effected:	None. No pyroxsulam related immobility and other sub-lethal adverse effects were noted during the exposure period of this study.

Data Evaluation Report on the acute toxicity of ATSA, a pyroxsulam (XDE-742) metabolite, to fresh water invertebrates - *Daphnia* sp.
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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study was stated to have generally conformed to current procedures described by:

- The Organisation for Economic Cooperation and Development (2004). *OECD Guideline for Testing of Chemicals No. 202, Daphnia* sp., Acute Immobilization Test. Adopted 13 April 2004;
- The Official Journal of the European Communities (1992). Annex to Commission *Directive 92/69/EEC, C.2. Acute Toxicity Test for Daphnia. Vol. 35, 29 December 1992*;
- U.S. Environmental Protection Agency. *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guideline 72-2 (referenced in the study report as, U.S. Environmental Protection Agency (1985). Hazard Evaluation Division: Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. Washington D.C.); and
- The U.S. EPA Standard Evaluation Procedure, "Acute Toxicity Test for Freshwater Invertebrates" (referenced in the study report as, U.S. Environmental Protection Agency (1982). *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guidelines 72-2. EPA 540/9-82-020, Washington, D.C.

Guidelines appear to have been generally followed with some deviations (acclimation issues, dilution water parameters, organic loading and provision of raw data) reported (see relevant text entries below and also the deficiencies or deviations from the guidelines and the relevant guideline requirements table on page 16 of this DER.

COMPLIANCE:

All aspects of testing were stated to have been conducted following:

- The OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17;
- The European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004); and
- Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

A signed and dated Compliance with Good Laboratory Practice Standards statement for the study was provided.

A signed and dated Quality Assurance Statement for the study was provided.

A signed and dated Statement of No Data Confidentiality Claims for the study was provided.

A. MATERIALS:

1. Test Material ATSA metabolite of pyroxsulam

Description: Solid

Lot No. /Batch No. : 035298-95

Purity: 100%

Stability of Compound Under Test Conditions:
Measured concentrations of ATSA Metabolite of pyroxsulam at test initiation and termination were within ± 20% of nominal concentrations (see page 10 of this DER for the measured concentrations reported), taken as indicating the material was stable throughout the duration of the 48-hour exposure period.

Storage conditions of test chemicals:
Information was not located in the study report. The company study profile template (Marino, 2006) reports that ambient room temperature storage conditions were used.

Physicochemical properties of ATSA metabolite of pyroxsulam

Parameter	Values	Comments
Water solubility at 20°C	Not available	The company's study profile template (Marino, 2006) stated that physicochemical properties of ATSA, a metabolite of pyroxsulam, were not available at the time of publication of the company's study profile template document.
Vapour pressure	Not available	
UV absorption	Not available	
pKa	Not available	
Kow	Not available	

2. Test organism:

Species: *Daphnia magna*
Age at test initiation: <24 hour old instars
Source: In-house cultures initially obtained from New England Bioassay, Inc., Manchester, Connecticut.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
A probe study was reported as, " … conducted between 10 January and 12 January 2006. One replicate of ten daphnids per test concentration was exposed to nominal concentrations of 0 (water control), 12.0, 60.0, and 120 mg ATSA Metabolite of XDE-742/L, over a 48-hour static exposure period. Following 48-hours of exposure, no daphnid immobility or changes in behaviour or physical appearance were observed in the water control or any of the treatment levels. Analytical chemistry confirmation of the day 0 test solution concentrations indicated that test solutions were dosed correctly. Percent of target values for the measured test concentrations ranged from approximately 93-96%. Based on the above information, the definitive study was conducted as a limit test with *Daphnia magna* exposed to a nominal concentration of 120 mg ATSA Metabolite of XDE-742/L."

b) Definitive Study

In the following table's Criteria column, entries in italics are those given in the PMRA's Draft Evaluation Report template for acute toxicity to the freshwater invertebrate, *Daphnia magna*. In its examination of the initial drafts of the aquatic invertebrate DERs, the PMRA advised (email of 3/07/2007) that the criteria in the templates were understood to have come from old US guidelines and that failure to comply with these template requirements would not be a deficiency. Provided the equivalent and more recent OPPTS and/or OECD guideline requirements are met, this is agreed with.

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation: Period:	In-house culture. Not specifically stated but the day before they were tested, the instars were obtained from daphnids which had had at least 3 broods.	See Table 5, Identified deficiencies or deviations from the guidelines and the relevant guideline requirements on page 16 of this DER. *EPA requires 7 day minimum acclimation period*
Conditions: (same as test or not)	Conditions considered equivalent because: The same water (adjusted [for hardness] laboratory dilution water, ALDW) was used for culturing and testing the daphnids. Rearing conditions were: Illumination 2050 ± 350 lux (in tests1950-2270 lux used), 16 hour light/8 hour dark photoperiod (also used in test), temperature 18-22°C (19-21°C in continuous monitoring in the test).	Requirement considered met.
Feeding:	During rearing, daphnids were typically fed a mixed diet of *Pseudokirchneriella subcapitata*, a freshwater green alga (formerly known as *Selenastrum capricornutum*) and YCT (yeast, Ceraphyll, and trout chow suspension) five times weekly. Daphnids were not fed during the test.	Requirement considered met. *EPA requires no feeding during study*
Health: (any mortality observed)	No specific comment identified in the study report. Rearing of at least 3 broods and the 100% survival of the control instars in the test indicates the daphnids used were healthy.	Requirement considered met.
Duration of the test	48 hours	Requirement met. *(EPA requires 96 hours, except daphnids which are 48 hours)*
Test conditions: Static/flow through	Static	Requirement met.

Parameter	Details	Remarks *Criteria*
Type of dilution system- for flow through method. Flow rate Renewal rate for static renewal	Not relevant for a static system. Not relevant for a static system. Not relevant for a static system	*EPA requires consistent flow rate of 5 – 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period)*
Aeration, if any	Laboratory dilution water was aerated for approximately 24 hours before use. No indication was provided that aeration of the test vessels occurred during the exposure period.	Requirement considered met.
Test vessel: Material: *(glass/stainless steel)* Size: Fill volume:	 Glass beakers (loosely covered) 250 mL ~200 mL	Requirement considered met. *(EPA requires: size 20 mL or 3.9 L fill 200 mL)*
Source of dilution water	Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water was obtained from the upper Saginaw Bay of Lake Huron off Whitestone Point and was limed and flocculated with ferric chloride.	Requirement considered met. *(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water)* The water was pumped to the laboratory prior to municipal treatment for human consumption. Before use in the laboratory, the water was sand-filtered, pH-adjusted with gaseous CO_2, carbon-filtered, and UV-irradiated. The water used for culturing and testing of daphnids (referred to as adjusted lab dilution water or ALDW) was prepared by adjusting the source water (LDW) to a target hardness of approximately 170 mg/L as $CaCO_3$. After adjusting hardness, the water was autoclaved at 250°F (121°C) and 18 psi for 30 minutes, cooled, and aerated for approximately 24 hours before use.
Water parameters:		See Table 5, Identified deficiencies or deviations from the guidelines and the relevant guideline requirements on page 16 of this DER with respect to OC, OP and PCB concentrations.

Parameter	Details	Remarks *Criteria*
Hardness	160 mg $CaCO_3$/L in control water and in 121 mg/L test solution (mean measured).	*hardness:* *EPA requires 40 - 48 mg/L as $CaCO_3$* OECD 202 refers a total hardness of 140-250 mg/L. . US EPA OPPTS 850.1010 refers to water quality parameters of a maximum hardness of 180 mg/L.
pH	Controls: 7.7 (day 0) and 7.6-7.7 (day 2); Test solution (121 mg ATSA metabolite of pyroxsulam/L, mean measured value): 6.6 (day 0) and 7.4 (day 2).	The pH range exceeds the US EPA range limits specified in the template (see below) but was within the OECD range of 6 to 9. US EPA OPPTS 850.1010 does not state a range but requires the pH to be measured at the start and end of the test *pH:* *EPA requires 7.2 - 7.6* The pH requirement is considered met.
Dissolved oxygen	Control: 8.5 (day 0) and 8.7 (day 2) mg/L; Test solution (121 mg ATSA metabolite of pyroxsulam/L, mean measured value): 8.6 (day 0) and 8.7-8.8 (day 2) mg/L. % Saturation: 96-99% (8.5-8.8 mg/L)	*Dissolved oxygen:* *EPA requires Static: 60% during 1st 48 hr and 40% during 2nd 48 hr Flow-through: 60%)* US EPA 850.1010 requires dissolved oxygen content to between 60 and 105 percent saturation. OECD 202 states that the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels.

Parameter	Details	Remarks *Criteria*
Temperature	All individual control and test solutions were maintained at 21°C over the 2 days of exposure. Continuous monitoring in a surrogate vessel reported a temperature range of 19-21°C over the exposure period.	*Temperature:* *EPA requires 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C;* OECD requires range of 18-22°C (±1°C)
Total organic carbon	390 µg/L	OECD 202 and US EPA OPTTS 850.1010 refers to dilution /testing water having, *inter alia*, a maximum TOC of 2.0 mg/L, a maximum particulate matter concentration of 20.0 mg/L.
Particulate matter	2000 µg/L (as total suspended solids)	OECD 202 and US EPA OPTTS 850.1010 refers to dilution water having, *inter alia*, a maximum particulate matter concentration of 20.0 mg/L.
Metals	Table of inorganic analyses identified most metallic ions were below their relevant detection levels as were anions such as bromide, chloride, nitrate etc. Where measurable concentrations were found (e.g. Al^{3+}, Ca^{2+}, Cl^- etc.), they were not identified as of concern.	Metals: OECD 202 says measurements of heavy metals should be made. Levels of inorganic residues reported are possibly consistent with the source of the water and, based on absence of immobility or sub-lethal effects in the control daphnids, not considered to have adversely affected the study.
Pesticides	A table of analysis of selected organic species and pesticides in laboratory water indicated all analytes measured were below their relevant limits of detection (which ranged from 0.25 to 5 µg/L).	While all selected organic species and pesticides analysed were below their relevant limits of detection, it is not possible to know if the sum of the organophosphorus pesticides and the total organochlorine pesticides plus polychlorinated biphenyls are below the maxima set by OPPTS 850.1010 for these parameters, i.e. 50 ng/L in both cases. Again, absence of immobility or sub-lethal effects in the control daphnids indicates that the levels present had not adversely affected the study.

Parameter	Details	Remarks *Criteria*
Chlorine	Below detection limit of 20 µg/L	OECD 202 refers to a residual chlorine level of <10 µg/L in acceptable dilution water. US EPA OPTTS 850.1010 refers to testing water having, *inter alia*, a residual chlorine content of <3 µg/L.
Intervals of water quality measurement	Dissolved oxygen, pH, and temperature data were recorded from the bulk treatment and control solutions at test initiation and from all test vessels at test termination. Water temperature was continuously monitored with a minimum/maximum thermometer placed in a surrogate test vessel and the range recorded daily. Light intensity was measured at each test vessel location on day 0. Water quality parameters, such as hardness, alkalinity, conductivity, and residual chlorine were measured from the day 0 bulk control water and treatment solutions. Both Laboratory Dilution Water (LDW) and adjusted laboratory dilution water (ALDW) water were typically monitored weekly for pH, alkalinity, conductivity, hardness, and residual chlorine. Periodically, the LDW (source water) was monitored for total organic carbon (TOC), total suspended solids (TSS), and selected inorganic and organic compounds.	ALDW adjusted for hardness, autoclaved and aerated prior to use. Requirement considered met.
Number of replicates: Control (dilution water): Solvent control: Treatments:	 3 Not applicable. No solvent used. 3	Requirement met. US EPA OPPTS 850.1010 refers to two or more replicates.
Number of organisms per replicate: Control (dilution water): Solvent control:	 10 Not applicable	See Table 5, Identified deficiencies or deviations from the guidelines and the relevant guideline requirements on page 16 of this DER with respect to biomass. *(EPA/OECD require 5 treatment*

<table>
<tr><th>Parameter</th><th>Details</th><th>Remarks
Criteria</th></tr>
<tr><td>Treatments:</td><td>10
(Control and one test concentration only tested – i.e. a limit test).
With 10 daphnids/~200 mL of test solution, the loading (biomass) would be 50 daphnids/L.</td><td>levels plus control
EPA requires a minimum of 20 daphnid per treatment. Biomass loading rate for static 0.8 g/L at 17oC, 0.5 g/L at > 17oC; flow-through: 1 g/L/day).
US EPA OPPTS 850.1010 refers to loading not exceeding 40 daphnids/L of test solution in the static system.</td></tr>
<tr><td><u>Treatment concentrations</u>:

Nominal:
Measured:</td><td>120 mg ATSA metabolite of pyroxsulam/L
Summary of results from the HPLC/UV analyses of test solutions of ATSA metabolite of pyroxsulam:

<table>
<tr><th rowspan="2">Test vessel identity</th><th colspan="2">Measured ATSA concentration, mg/L (% of nominal)</th><th rowspan="2">Mean measured concen-tration[a] mg/L (% nominal).</th></tr>
<tr><th>Day 0</th><th>Day 2</th></tr>
<tr><td colspan="4">ALWD Controls</td></tr>
<tr><td>A, day 0 bulk sample</td><td><LLQ[b]</td><td><LLQ</td><td rowspan="3">Not applicable</td></tr>
<tr><td>B</td><td>Not measured</td><td><LLQ</td></tr>
<tr><td>C</td><td>Not measured</td><td><LLQ</td></tr>
<tr><td colspan="4">Nominal: 120 mg ATSA metabolite of pyroxsulam/L</td></tr>
<tr><td>A, day 0 bulk sample</td><td>122[c] (102%)</td><td>120 (100%)</td><td>121</td></tr>
<tr><td>B</td><td>Not measured</td><td>121 (101%)</td><td>121</td></tr>
<tr><td>C</td><td>Not measured</td><td>120 (100%)</td><td>120</td></tr>
<tr><td>Mean:</td><td>122 (102%)</td><td>120 (100%)</td><td>121 (101%)</td></tr>
</table>
a. Mean concentration of the day 0 and day 2 concentrations.
b. LLQ = Lowest level quantified (0.252 mg ASTA metabolite of pyroxsulam/L ALDW).</td><td>Requirement considered met as the study was a limit test conducted at only one test concentration.

(EPA requires a geometric series with each concentration being at least 60% of the next higher one)

The measured results show 100 to 102% recoveries of the nominal 120 mg/L occurred. Such results indicate that the ASTA metabolite of pyroxsulam was stable in the test solutions over the 48 hours of the exposure period.</td></tr>
</table>

Parameter	Details	Remarks *Criteria*
Solvent (type, percentage, if used)	Solvent not used.	Requirement met. *(EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests)*
Lighting	The photoperiod was set at 16 hours of light/8 hours of dark per day. Light intensity (raw data were not presented) ranged from 1950-2270 lux.	Requirement considered met. *(EPA requires 16 hours light, 8 hours dark; OECD : optional light-dark cycle or complete darkness).* OECD 202 and US EPA OPPTS 850.1010 recommend a 16 hours light and 8 hour dark cycle.
Recovery of chemical: Frequency of determination Level of Quantitation Level of Detection	 ATSA residues were determined at day 0 in the control and bulk dose solutions and at day 2 in the control and test solutions. The lowest concentration quantified was 0.252 mg ATSA metabolite of pyroxsulam/L of ALDW. Not located in the study report.	Requirement considered met. Typical chromatograms of a control ALDW sample, a 0.229 mg ATSA metabolite of pyroxsulam/L standard and of a nominal 120 mg ATSA metabolite of pyroxsulam/L test solution were presented as was a calibration curve of concentration versus peak area.
Positive control {if used, indicate the chemical and concentrations}	A positive control was not used.	Requirement considered met.
Other parameters, if any	None identified.	Requirement considered met.

2. Observations:

Table 2. Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sub-lethal effects	Daphnid immobility or change in behaviour or appearance (sub-lethal effects). Immobility was defined as the inability to swim within 15 seconds after gentle agitation of the test container.	Requirement considered met. OECD 202 and US EPA OPPTS 850.1010 refer to immobilisation as the effect measured but also state that any adverse effects, including abnormal behaviour or appearance, should be reported.
Observation intervals	Biological observations were made at 24 and 48 hours.	Requirement considered met. OECD 202 and US EPA OPPTS 850.1010 refer to checking for immobilized daphnids at 24 and 48 hours after the beginning of the test. OECD 202 also refers to checking for any abnormal behaviour or appearances at those times.
Water quality was acceptable (Yes/No)	Yes, based on the absence of immobility and other adverse effects in the control solutions.	Requirement considered met. OECD 202 and US EPA OPPTS 850.1010 refer to dilution water being acceptable as dilution water if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress.
Were raw data included?	No raw data relating to the biological observations was provided. Instead, tabulated data were presented. The data, protocol, protocol changes/revisions, and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.	OECD 202 makes no comment on supply of raw data and allows for presentation in a summarised, tablular form. The absence of raw data is not considered a deficiency even though US OPPTS 850.1010 states that the sponsor must submit to the EPA all data developed by the test that are suggestive or predictive of acute toxicity and all concomitant gross toxicological manifestations. This decision on the absence of a deficiency is on the basis of advice from the US EPA that tabulated results are considered sufficient as they allow recalculation of dose response if necessary.
Other observations, if any	Evidence of incomplete dissolution of the test material was absent and the solution clear following preparation of this treatment solution.	Requirement considered met.

II. RESULTS AND DISCUSSION

A. MORTALITY:

No daphnid immobility was seen in the daphnids in the control or limit test exposure solutions (Table 3). Note that in this study, the effect criterion was set as immobility (rather than mortality *per se*) with this defined as the inability to swim within 15 seconds after gentle agitation of the test container.

Table 3. Effect of the ATSA metabolite of pyroxsulam on immobilisation of *Daphnia magna*.

Treatment (mg ATSA metabolite of pyroxsulam/L) [Mean measured (nominal) concentrations]	Total number of organisms (in three replicates)	Observation period			
		24 hours		48 hours	
		Number immobilised	% immbolisation	Number immobilised	% immbolisation
Control (dilution water only) 0 (<0.252) mg/L	30	0	0	0	0
Solvent control, if used	Solvent control not used.				
121 (120) mg/L	30	0	0	0	0
NOEC (24 and 48 hours, immobility)	121 mg ATSA metabolite of pyroxsulam/L (mean, analytically determined over 48 hours); 120 mg ATSA metabolite of pyroxsulam/L (nominal)				
EC50 (24 and 48 hours, immobility)	>121 mg ATSA metabolite of pyroxsulam/L (mean, analytically determined over 48 hours); >120 mg ATSA metabolite of pyroxsulam/L (nominal)				
Positive control, if used	Positive control not used.				

B. SUB-LETHAL TOXICITY ENDPOINTS:

Sub-lethal (daphnid behaviour or appearance) effect were not seen in the daphnids in the control or limit test exposure solutions (Table 4).

Table 4. Effect of the ATSA metabolite of pyroxsulam on sub-lethal endpoints (behaviour or appearance) - *Daphnia magna.*

Treatment (mg ATSA metabolite of pyroxsulam/L) Mean-measured (nominal) concentrations	Total number of organisms (in three replicates)	Observation period			
		24 hours		48 hours	
		Number with sub-lethal effects	% with sub-lethal effects	Number with sub-lethal effects	% with sub-lethal effects
Control (dilution water only 0 (<0.252) mg/L	30	0	0	0	0
Solvent control, if used	Solvent control not used.				
121 (120) mg/L	30	0	0	0	0
NOEC (24 and 48 hours, sub-lethal effects)	121 mg ATSA metabolite of pyroxsulam/L (mean, analytically determined over 48 hours); 120 mg ATSA metabolite of pyroxsulam/L (nominal)				
EC50 (24 and 48 hours, sub-lethal effects)	>121 mg ATSA metabolite of pyroxsulam/L (mean, analytically determined over 48 hours); >120 mg ATSA metabolite of pyroxsulam/L (nominal)				
Positive control, if used	Positive control not used.				

C. REPORTED STATISTICS:

The parameters analysed in the study were daphnid immobilisation and sub-lethal effects at 24 and 48 hours, concentration of ATSA metabolite of pyroxsulam in the test solutions at 0 and 48 hours and water parameters (pH, temperature and dissolved oxygen content) of the control and test solutions over 48 hours.

Due to the study design (i.e., limit test), the statistical evaluation of the biological data was reported as not attempted. The 24 and 48 hour EC_{50} values were stated to have both been empirically determined to be greater than the mean measured limit concentration tested (namely, 121 mg ATSA metabolite of pyroxsulam/L).

The 24 and 48 hour NOECs were reported as determined based on the mean measured limit concentration tested exhibiting no daphnid immobility or change in behaviour or appearance, (namely, 121 mg ATSA metabolite of pyroxsulam/L).

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Method:
The absence of immobilisation or adverse effects in the control and test solutions and the acceptability of the measured concentrations, pH, oxygen content and temperature values support the study report's decision not

to conduct a statistical analysis of the data.

The 24 and 48 hour NOECs and EC50s for immobilisation and adverse effects can be estimated from a visual inspection of the results presented for these parameters.

Statistical Method: Not conducted as a result of the study's results. Consequently, the 48 hours EC_{50} and the NOEC were not calculated by the reviewer using statistical methodology.

E. STUDY DEFICIENCIES:

The following deviations from guidelines were noted but not considered to have significantly affected the study's outcome on the basis of the absence of effects in the control daphnids:

Table 5. Identified deficiencies or deviations from the guidelines and the relevant guideline requirements.

Parameter	Study report result	US EPA OPPTS 850.1010 Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids, April 1996	OECD Guideline for Testing Chemicals, *Daphnia* sp., Acute Immobilisation Test, 202, adopted 13 April 2004
Acclimation period and observations during that time	Not specifically stated but the instars were obtained from daphnids which had had at least 3 broods.	Refers to "At the initiation of the test, daphnids which have been cultured and acclimated in accordance with the test design …" and to "Brood daphnids should be maintained in 100-percent dilution water at the test temperature for at least 48 h prior to the start of the test." Additionally, the data records of the culture, acclimation, and test temperatures must be submitted by the sponsor to the EPA. Also, "During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage, and mortality." Based on US EPA advice, adequate acclimatisation is considered to have occurred.	The stock animals must be maintained in culture conditions (light, temperature, medium) similar to those to be used in the test.
Dilution water parameters			
Pesticides	A table of analysis of	Water quality parameters	Water quality parameters

	selected organic species and pesticides in laboratory water indicated all analytes measured were below their relevant limits of detection (which ranged from 0.25 to 5 µg/L).	include requirements that total organophosphorus pesticides do not exceed 50 ng/L and that total organochlorine pesticides plus polychlorinated biphenyls (PCBs) do not exceed 50 ng/L.	include requirements that total organophosphorus pesticides do not exceed 50 ng/L and that total organochlorine pesticides plus polychlorinated biphenyls (PCBs) do not exceed 50 ng/L.
Number of organisms per replicate: Loading (biomass)	10 daphnid/~200 mL of solution, equivalent to ~50 daphnids/L	US EPA OPPTS 850.1010 refers to loading not exceeding 40 daphnids/L of test solution in the static system.	At least 2 ml of test solution should be provided for each animal (i.e. a volume of 10 ml for five daphnids per test vessel).

F. REVIEWER'S COMMENTS:

This daphnid acute toxicity study was conducted as a limit test with a nominal concentration of 120 mg of 100% ASTA metabolite of pyroxsulam/L with a mean-measured concentration over 48 hours of 121 mg ASTA metabolite of pyroxsulam/L. As the 48 hour EC50s for immobilisation and sub-lethal effects were both >121 mg ASTA metabolite of pyroxsulam/L (based on mean, measured concentrations over 48 hours), the ASTA metabolite of pyroxsulam is considered practically non-toxic to *D. magna* instars (48 hour EC50 >100 mg /L).

Although deviations from the guidelines were identified, these were considered not to have affected either the validity or the results of the study with the absence of immobility or other sub-lethal effects in the control showing the test conditions were acceptable. Consequently, the validity criteria for OECD 203 (adopted 17.07.92) and US EPA OPPTS 850.1075, namely not more than 10% mortality in the controls and acceptable dissolved oxygen contents (>3 mg/L in the control and test vessels at the end of the study for the OECD guideline and between 60 and 105% saturation for the US EPA standard), were considered to have been met by the study.

The in-life portion of the definitive toxicity test was conducted from 7 February to 9 February 2006.

The PMRA reviewer agrees with the conclusions of the reviewer from the Australian Government Department of the Environment and Water Resources. This study is acceptable to the PMRA.

G. CONCLUSIONS:

This study is considered acceptable. The 48 hour acute static toxicity (limit test) study with *Daphnia magna* instars resulted in a 48 hour EC50 of the ASTA metabolite of pyroxsulam (as 100% active constituent) to the daphnids of >120 mg/L based on the nominal test concentration and >121 mg ASTA metabolite of pyroxsulam/L for immobilisation based on mean measured concentration of the test substance over 48 hours.

The 48 hour EC50 for sub-lethal effects (behaviour and appearance) in the daphnid instars was determined to have the same value as reported for the 48 hour EC50 for immobilisation, i.e. >120 mg ASTA metabolite of pyroxsulam/L based on nominal concentration of the test substance and >121 mg/L based on the mean analytically determined concentration.

The 48 h NOECs for immobilisation and sub-lethal effects were both 120 mg ASTA metabolite of pyroxsulam/L (nominal concentrations) or 121 mg ASTA metabolite of pyroxsulam/L based on the mean analytically determined concentration.

Based on the results of this study, the ASTA metabolite of pyroxsulam would be classified as practically non-toxic to *Daphnia magna* in accordance with the classification system of the Australian Government Department of the Environment and Water Resources (EC50 > 100 mg/L) and that of the US EPA..

III. REFERENCES:

Note: for the purpose of this parallel process work, references to standard guidelines or methodologies have been included at this time in the list of references.

Dow AgroSciences Test Substance Distribution Certificate. TSN105493, Dow AgroSciences LLC, Indianapolis, Indiana.

Environmental Protection Agency-FIFRA GLPS; Title 40 CFR Part 160-Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule.

European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004).

Madsen S (2005). Certificate of Analysis for Test Substances: FA & PC Number 053486, Dow AgroSciences LLC, Indianapolis, Indiana. 19 December 2005.

Marino T A (2006). Study Profile Template (SPT) for ATSA Metabolite of XDE-742: An Acute Toxicity Study with the Daphnid, *Daphnia Magna.* Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan 48674. Study ID 061005.SPT. Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268. 17 March 2006. Unpublished report.

OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17.

Official Journal of the European Communities (1992). Annex to Commission Directive 92/69/EEC, C.2. Acute Toxicity Test for *Daphnia.* Vol. 35, 29 December 1992.

Organisation for Economic Cooperation and Development (2004). *OECD Guideline for Testing of Chemicals No. 202, Daphnia* sp., Acute Immobilization Test. Adopted 13 April 2004.

U.S. Environmental Protection Agency (1982). *Pesticide Assessment Guidelines,* Subdivision E Hazard Evaluation: Wildlife and Aquatic Organisms, Guidelines 72-2. EPA 540/9-82-020, Washington, D.C.

U.S. Environmental Protection Agency (1985). Hazard Evaluation Division: Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Invertebrates. EPA-540/9-85-005. Washington D.C.

Approved 04/01/01 C. K.